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SUSCEPTIBILITY

In Vitro Activity of Imipenem-Relebactam against Gram-Negative ESKAPE Pathogens Isolated by Clinical Laboratories in the United States in 2015 (Results from the SMART Global Surveillance Program)

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ABSTRACT Relebactam (formerly MK-7655) is an inhibitor of class A and C β-lactamases, including *Klebsiella pneumoniae* carbapenemase (KPC), and is currently in clinical development in combination with imipenem-cilastatin. Using Clinical and Laboratory Standards Institute (CLSI)-defined broth microdilution methodology, we evaluated the *in vitro* activities of imipenem-relebactam, imipenem, and seven routinely tested parenteral antimicrobial agents against Gram-negative ESKAPE pathogens (including *Klebsiella pneumoniae*, $n = 689$; *Acinetobacter baumannii*, $n = 72$; *Pseudomonas aeruginosa*, $n = 845$; and *Enterobacter* spp., $n = 399$) submitted by 21 clinical laboratories in the United States in 2015 as part of the SMART (Study for Monitoring Antimicrobial Resistance Trends) global surveillance program. Relebactam was tested at a fixed concentration of 4 μg/ml in combination with doubling dilutions of imipenem. Imipenem-relebactam MICs were interpreted using CLSI imipenem breakpoints. The respective rates of susceptibility to imipenem-relebactam and imipenem were 94.2% (796/845) and 70.3% (594/845) for *P. aeruginosa*, 99.0% (682/689) and 96.1% (662/689) for *K. pneumoniae*, and 100% (399/399) and 98.0% (391/399) for *Enterobacter* spp. Relebactam restored imipenem susceptibility to 80.5% (202/251), 74.1% (20/27), and 100% (8/8) of isolates of imipenem-nonsusceptible *P. aeruginosa*, *K. pneumoniae*, and *Enterobacter* spp. Relebactam did not increase the number of isolates of *Acinetobacter* spp. susceptible to imipenem, and the rates of resistance to all of the agents tested against this pathogen were >30%. Further development of imipenem-relebactam is warranted given the demonstrated ability of relebactam to restore the activity of imipenem against current clinical isolates of *Enterobacteriaceae* and *P. aeruginosa* that are nonsusceptible to carbapenems and its potential as a therapy for treating patients with antimicrobial-resistant Gram-negative infections.

KEYWORDS SMART, surveillance, imipenem, relebactam, ESKAPE pathogens

Carbapenems are broad-spectrum antibacterial agents that provide reliable clinically effective therapy for infections arising from aerobic and anaerobic Gram-positive and Gram-negative bacteria. They are generally reserved for the treatment of serious nosocomial infections and are frequently used as the agents of last resort. Carbapenems demonstrate stability against many β-lactamases, including class A extended-spectrum β-lactamases (ESBLs) and class C β-lactamases (AmpC). The primary mechanisms of resistance to carbapenems demonstrated by Gram-negative bacteria include

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carbapenemase production, impaired outer membrane permeability resulting from the reduced expression of particular outer membrane proteins (Opr proteins and porins), efflux across the outer membrane, or a combination of these mechanisms. *Klebsiella pneumoniae* carbapenemase (KPC)-type carbapenemases, a group of plasmid-encoded class A β-lactamases, initially emerged in the northeastern United States around the year 2000, have spread globally, and are increasingly important purveyors of carbapenem resistance in *Enterobacteriaceae* worldwide. Carbapenems are also hydrolyzed by class B metallo-β-lactamases (e.g., NDM, IMP, and VIM) and class D β-lactamases (e.g., OXA-type). Carbapenem resistance in *Pseudomonas aeruginosa* most commonly occurs as the result of the downregulation of the porin protein OprD in combination with the production of the intrinsic chromosomally encoded AmpC β-lactamase (*Pseudomonas*-derived cephalosporinase [PDC]) (1–4).

The addition of a β-lactamase inhibitor (e.g., clavulanate, sulbactam, or tazobactam) to restore the activity of a compromised β-lactam (e.g., amoxicillin, ampicillin, or piperacillin) has been demonstrated to be a clinically effective strategy in antimicrobial development, because β-lactamases are one of the primary mechanisms underlying β-lactam nonsusceptibility in Gram-negative bacilli (5). However, clavulanate, sulbactam, and tazobactam only inhibit selected class A enzymes, excluding KPC-type carbapenemases, and generally have a minimal effect on AmpC β-lactamases, although some AmpC β-lactamases are inhibited by sulbactam or tazobactam (6, 7). Relebactam (formerly MK-7655) is a novel, non-β-lactam bicyclic diazabicyclooctane β-lactamase inhibitor that is structurally related to avibactam and, like avibactam, is active *in vitro* against class A β-lactamases, including KPC-type carbapenemases, and class C β-lactamases (8). While avibactam has been studied primarily in combination with cephalosporins and aztreonam (and has been approved for use with ceftazidime), relebactam has been combined with the carbapenem-renal dehydropeptidase-I inhibitor, imipenem-cilastatin, to restore imipenem's clinical activity against KPC-producing *K. pneumoniae*, other carbapenem-resistant *Enterobacteriaceae*, and *P. aeruginosa* that demonstrate carbapenem resistance due to impermeability arising from porin loss combined with AmpC expression (8, 9). In particular, imipenem has been identified as an excellent partner for relebactam to treat pseudomonal infections because neither imipenem nor relebactam is subject to efflux in *P. aeruginosa* (9, 10).

The acronym ESKAPE defines a group of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* spp.) that are responsible for a majority of antimicrobial-resistant hospital-associated infections (11, 12). ESKAPE pathogens are known to "escape" the effects of currently marketed antimicrobial agents, are frequently multidrug resistant, and are associated with poor patient outcomes, because patients infected with ESKAPE pathogens often receive inappropriate empirical antimicrobial therapy that leads to unfavorable clinical outcomes, high case fatality rates, and opportunities for the pathogen to spread to other patients (11, 13–16). Gram-negative ESKAPE pathogens act as important reservoirs and transmitters of resistance and are responsible for the increased reporting of antimicrobial-resistant nosocomial infections worldwide (11–13, 16). There are very few new antimicrobial agents in development to treat Gram-negative ESKAPE pathogens despite the well-recognized need (11–13, 17).

The intention of the current study was to determine the *in vitro* activity of imipenem-relebactam, a novel carbapenem-carbapenemase inhibitor combination, against a current collection of Gram-negative ESKAPE pathogens isolated from patients with intra-abdominal, urinary tract, and lower respiratory tract infections in the United States in 2015. The isolates tested in this study were collected as part of the Study for Monitoring Antimicrobial Resistance Trends (SMART) global surveillance program, which has monitored the *in vitro* antimicrobial susceptibility profiles of clinical isolates of aerobic and facultative Gram-negative bacilli collected by laboratories worldwide from patients with intra-abdominal (since 2002), urinary tract (since 2009), and lower respiratory tract (since 2015) infections (18).

RESULTS

For *P. aeruginosa*, 94.2% (796/845) and 70.3% (594/845) of isolates were susceptible to imipenem-relebactam and imipenem, respectively; 80.5% (202/251) of imipenem-nonsusceptible isolates of *P. aeruginosa* were rendered susceptible by the addition of relebactam (Table 1). The MIC_{90} for imipenem-relebactam (2 μg/ml) was 8-fold lower than for imipenem alone (16 μg/ml). Of the comparator agents, only amikacin showed *in vitro* activity (95.6% susceptible) comparable to imipenem-relebactam; susceptibilities of *P. aeruginosa* to all of the other agents tested were $<$80%. Figure 1a depicts the effect of relebactam on the distribution of MICs for imipenem against all 845 isolates of *P. aeruginosa* tested and shows that the modal MIC for imipenem dropped from 1 to $\leq$0.5 μg/ml in the presence of relebactam. Figure 1b shows the effect of relebactam on the distribution of MICs for imipenem against the 251 isolates of imipenem-nonsusceptible *P. aeruginosa* and that the modal MIC for imipenem dropped from 16 to 1 μg/ml in the presence of relebactam. For the 202 of 251 (80.5%) isolates of *P. aeruginosa* that were imipenem-nonsusceptible and susceptible to imipenem-relebactam, one isolate carried a VEB-type ESBL and the other 201 did not carry a β-lactamase other than the intrinsic chromosomally encoded *Pseudomonas*-derived cephalosporinase (PDC) common to this species. The other 49 isolates (nonsusceptible to both imipenem and imipenem-relebactam) comprised two isolates carrying a VIM-type metallo-β-lactamase and 47 isolates carrying only PDC (Table 2).

For *K. pneumoniae*, 99.0% (682/689) and 96.1% (662/689) of isolates were susceptible to imipenem-relebactam and imipenem, respectively; 74.1% (20/27) of imipenem-nonsusceptible isolates of *K. pneumoniae* were rendered susceptible by the addition of relebactam (Table 1). Of the comparator agents, only amikacin showed *in vitro* activity (97.5% susceptible) comparable to those of imipenem-relebactam and imipenem; the other agents tested had susceptibilities of approximately 90%. Figure 1 shows the effect of relebactam on the distribution of MICs for imipenem against all 689 isolates of *K. pneumoniae* tested (Fig. 1c) and against the 27 isolates of imipenem-nonsusceptible *K. pneumoniae* (Fig. 1d). Because of the small proportion of imipenem-nonsusceptible isolates among all *K. pneumoniae* (3.9%), the imipenem MIC distributions were similar with and without relebactam (Fig. 1c) and the already high imipenem susceptibility of 96.1% increased by only an additional 2.9% (Table 1). However, for the 27 isolates that were nonsusceptible to imipenem, the modal MIC decreased at least 16-fold from 8 to $\leq$0.5 μg/ml. Among the imipenem-nonsusceptible *K. pneumoniae* isolates, 74.1% (20/27) carried KPC and were susceptible to imipenem-relebactam; the other seven isolates that were nonsusceptible to imipenem-relebactam carried OXA-48-type carbapenemases, metallo-β-lactamases (VIM), or the class A carbapenemase GES-20 (Table 2).

For *Enterobacter* spp., 100% (399/399) and 98.0% (391/399) of isolates were susceptible to imipenem-relebactam and imipenem, respectively; 100% (8/8) of imipenem-nonsusceptible isolates of *Enterobacter* spp. were rendered susceptible by the addition of relebactam (Table 1). Of the comparator agents, only amikacin (99.3% susceptible) and levofloxacin (96.2% susceptible) showed *in vitro* activities comparable to those of imipenem-relebactam and imipenem; the other agents tested had percent susceptibilities of approximately 90% (cefepime) or $\leq$80% (all other agents). Figure 1 shows the effect of relebactam on the distribution of MICs for imipenem against all 399 isolates of *Enterobacter* spp. (Fig. 1e) and against eight isolates of imipenem-nonsusceptible *Enterobacter* spp. (Fig. 1f). The eight imipenem-nonsusceptible isolates were all susceptible to imipenem-relebactam and included one isolate with KPC and seven isolates in which no acquired β-lactamase was identified (Table 2).

Against *A. baumannii*, relebactam did not increase the number of isolates susceptible to imipenem, and the rates of resistance to all agents tested, including imipenem-relebactam and amikacin, were $>$30%. All of the isolates of imipenem-nonsusceptible *A. baumannii* were also nonsusceptible to imipenem-relebactam.

TABLE 1 *In vitro* activity of imipenem-relebactam and comparative antimicrobial agents against Gram-negative ESKAPE pathogens[a]

Organism (no. of isolates)	Antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	Percentage of isolates		
					Susceptible	Intermediate[b]	Resistant
All *P. aeruginosa* (845)	Imipenem-relebactam	0.5	2	0.06 to >32	94.2	3.2	2.6
	Imipenem	1	16	≤0.5 to >32	70.3	5.1	24.6
	Amikacin	≤4	8	≤4 to >32	95.6	2.1	2.3
	Aztreonam	8	>16	≤1 to >16	64.1	13.6	22.3
	Cefepime	4	32	≤1 to >32	75.0	13.5	11.5
	Ceftazidime	4	32	≤0.5 to >32	77.3	5.3	17.4
	Ceftriaxone	>32	>32	≤1 to >32	NA[c]	NA	NA
	Levofloxacin	1	>4	≤0.5 to >4	67.1	8.1	24.9
	Piperacillin-tazobactam	8	>64	≤2 to >64	70.1	12.5	17.4
Imipenem-nonsusceptible *P. aeruginosa* (251)	Imipenem-relebactam	2	4	0.25 to >32	80.5	10.8	8.8
	Imipenem	16	32	4 to >32	0	17.1	82.9
	Amikacin	≤4	16	≤4 to >32	91.2	3.6	5.2
	Aztreonam	16	>16	≤1to >16	33.1	20.7	46.2
	Cefepime	16	>32	≤1 to >32	46.6	25.1	28.3
	Ceftazidime	8	>32	≤0.5 to >32	52.6	10.0	37.5
	Ceftriaxone	>32	>32	≤1 to >32	NA	NA	NA
	Levofloxacin	>4	>4	≤0.5 to >4	34.7	12.4	53.0
	Piperacillin-tazobactam	32	>64	≤2 to >64	40.2	21.1	38.7
All *K. pneumoniae* (689)	Imipenem-relebactam	0.12	0.5	≤0.03 to 4	99.0	0.7	0.3
	Imipenem	≤0.5	≤0.5	≤0.5 to >32	96.1	0	3.9
	Amikacin	≤4	≤4	≤4 to >32	97.5	1.0	1.5
	Aztreonam	≤1	16	≤1 to >16	89.7	0.2	10.2
	Cefepime	≤1	4	≤1 to >32	89.6	2.8	7.7
	Ceftazidime	≤0.5	8	≤0.5 to >32	89.8	1.0	9.1
	Ceftriaxone	≤1	32	≤1 to >32	88.4	0.3	11.3
	Levofloxacin	≤0.5	2	≤0.5 to >4	90.9	1.0	8.1
	Piperacillin-tazobactam	4	16	≤2 to >64	90.9	2.3	6.8
Imipenem-nonsusceptible *K. pneumoniae* (27)	Imipenem-relebactam	0.25	2	0.06 to 4	74.1	18.5	7.4
	Imipenem	8	32	4 to >32	0	0	100
	Amikacin	16	>32	≤4 to >32	55.6	22.2	22.2
	Aztreonam	>16	>16	>16 to >16	0	0	100
	Cefepime	32	>32	8 to >32	0	3.7	96.3
	Ceftazidime	>32	>32	32 to >32	0	0	100
	Ceftriaxone	>32	>32	32 to >32	0	0	100
	Levofloxacin	>4	>4	1 to >4	11.1	0	88.9
	Piperacillin-tazobactam	>64	>64	>64 to >64	0	0	100
Enterobacter spp. (399)	Imipenem-relebactam	0.25	0.5	≤0.03 to 1	100	0	0
	Imipenem	≤0.5	1	≤0.5 to 32	98.0	1.5	0.5
	Amikacin	≤4	≤4	≤4 to >32	99.3	0	0.8
	Aztreonam	≤1	>16	≤1 to >16	78.5	2.3	19.3
	Cefepime	≤1	2	≤1 to >32	91.7	7.0	1.3
	Ceftazidime	≤0.5	>32	≤0.5 to >32	79.0	1.3	19.8
	Ceftriaxone	≤1	>32	≤1 to >32	74.2	2.8	23.1
	Levofloxacin	≤0.5	≤0.5	≤0.5 to >4	96.2	1.0	2.8
	Piperacillin-tazobactam	4	64	≤2 to >64	80.2	11.0	8.8
Imipenem-nonsusceptible *Enterobacter* spp. (8)[d]	Imipenem-relebactam	–	–	0.12 to 1	8 of 8	0 of 8	0 of 8
	Imipenem	–	–	2 to 32	0 of 8	6 of 8	2 of 8
	Amikacin	–	–	≤4 to ≤4	8 of 8	0 of 8	0 of 8
	Aztreonam	–	–	≤1 to >16	5 of 8	0 of 8	3 of 8
	Cefepime	–	–	≤1 to 32	7 of 8	0 of 8	1 of 8
	Ceftazidime	–	–	≤0.5 to >32	6 of 8	0 of 8	2 of 8
	Ceftriaxone	–	–	≤1 to >32	5 of 8	1 of 8	2 of 8
	Levofloxacin	–	–	≤0.5 to ≤0.5	8 of 8	0 of 8	0 of 8
	Piperacillin-tazobactam	–	–	≤2 to >64	5 of 8	1 of 8	2 of 8
A. baumannii (72)	Imipenem-relebactam	4	>32	0.12 to >32	45.8	4.2	50.0
	Imipenem	8	>32	≤0.5 to >32	45.8	2.8	51.4
	Amikacin	≤4	>32	≤4 to >32	66.7	2.8	30.6
	Aztreonam	>16	>16	4 to >16	NA	NA	NA

(Continued on next page)

TABLE 1 (Continued)

Organism (no. of isolates)	Antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	Percentage of isolates		
					Susceptible	Intermediate[b]	Resistant
	Cefepime	16	>32	≤1 to >32	37.5	15.3	47.2
	Ceftazidime	32	>32	1 to >32	40.3	2.8	56.9
	Ceftriaxone	>32	>32	2 to >32	16.7	25.0	58.3
	Levofloxacin	>4	>4	≤0.5 to >4	37.5	2.8	59.7
	Piperacillin-tazobactam	>64	>64	≤2 - >64	31.9	9.7	58.3
Imipenem-nonsusceptible *A. baumannii* (39)	Imipenem-relebactam	32	>32	4 to >32	0	7.7	92.3
	Imipenem	32	>32	4 to >32	0	5.1	94.9
	Amikacin	16	>32	≤4 to >32	51.3	5.1	43.6
	Aztreonam	>16	>16	16 to >16	NA	NA	NA
	Cefepime	32	>32	2 to >32	7.7	20.5	71.8
	Ceftazidime	>32	>32	8 to >32	12.8	0	87.2
	Ceftriaxone	>32	>32	8 to >32	2.6	10.3	87.2
	Levofloxacin	>4	>4	≤0.5 to >4	2.6	5.1	92.3
	Piperacillin-tazobactam	>64	>64	≤2 to >64	2.6	5.1	92.3

[a]Pathogens were from isolates collected from patients with intra-abdominal, urinary tract, and lower respiratory tract infections in the United States included in the SMART global surveillance study in 2015.
[b]For *Enterobacteriaceae* tested against cefepime, the intermediate category is replaced by the "susceptible-dose dependent" category (14).
[c]NA, not applicable. MIC breakpoints are not published for the antimicrobial agent and organism combination (14).
[d]When the number of isolates was <10, MIC_{50} and MIC_{90} values are not reported (–). Instead of percentages, the numbers of susceptible, intermediate, and resistant isolates from the total number of isolates tested are shown.

DISCUSSION

The current study determined that most isolates of the Gram-negative ESKAPE pathogens *P. aeruginosa* (94.2% susceptible), *K. pneumoniae* (99.0% susceptible), and *Enterobacter* spp. (100% susceptible) were susceptible to imipenem-relebactam and that relebactam restored imipenem susceptibility to 80.5% (202/251), 74.1% (20/27), and 100% (8/8) of isolates of *P. aeruginosa*, *K. pneumoniae*, and *Enterobacter* spp. that were nonsusceptible to imipenem, respectively. Other investigators have also observed that relebactam restores the *in vitro* activity of imipenem against Gram-negative pathogens that are otherwise nonsusceptible to carbapenems (9, 19). The greatest impact of the addition of relebactam to imipenem has been reported for isolates of *K. pneumoniae* that harbor KPC-type carbapenemases and ESBLs, as well as for isolates of carbapenem-resistant *P. aeruginosa* that lack OprD and express AmpC β-lactamase (9, 19).

Livermore et al. reported that at a concentration of 4 μg/ml relebactam lowered imipenem MICs for imipenem-susceptible *P. aeruginosa* ($n = 8$) from mostly 1 to 2 μg/ml to 0.25 to 0.5 μg/ml (7/8 isolates), lowered MICs for imipenem-resistant OprD-deficient isolates of *P. aeruginosa* without metallo-β-lactamases ($n = 8$) from 16 to 64 μg/ml to 1 to 4 μg/ml, lowered MICs for imipenem-nonsusceptible AmpC-derepressed isolates of *P. aeruginosa* without metallo-β-lactamases ($n = 8$) from 2 to 32 μg/ml to 0.5 to 4 μg/ml, and lowered MICs for multidrug-resistant (including imipenem-resistant) isolates of *P. aeruginosa* from both cystic fibrosis and non-cystic fibrosis patients ($n = 16$) from 8 to 64 μg/ml to 2 to 16 μg/ml (9). In another study, Lapuebla and colleagues tested 490 isolates of *P. aeruginosa* and reported MIC_{50} and MIC_{90} values for imipenem of 2 and 16 μg/ml, respectively (19). With the addition of relebactam at a concentration of 4 μg/ml, the MIC_{50} and MIC_{90} values for the same isolate collection decreased by approximately 4-fold to 0.5 and 2 μg/ml, respectively, and the imipenem susceptibility rate increased from 70 to 98%. Among the 144 isolates of *P. aeruginosa* in that collection that were imipenem-nonsusceptible, the addition of relebactam produced MIC_{50} and MIC_{90} values of 1 and 2 μg/ml, respectively (19). Lapuebla et al. also reported on 30 previously characterized isolates of *P. aeruginosa* devoid of carbapenemases and observed that imipenem MICs were lowered by the addition of relebactam at a concentration of 4 μg/ml for isolates with depressed *oprD* expression with and without increased *ampC* expression (19). Livermore et al. indicated that both imipenem and relebactam were poor substrates for efflux in *P. aeruginosa* and speculated that relebactam potentiates the activity of imipenem against *P. aeruginosa* by inhibiting the

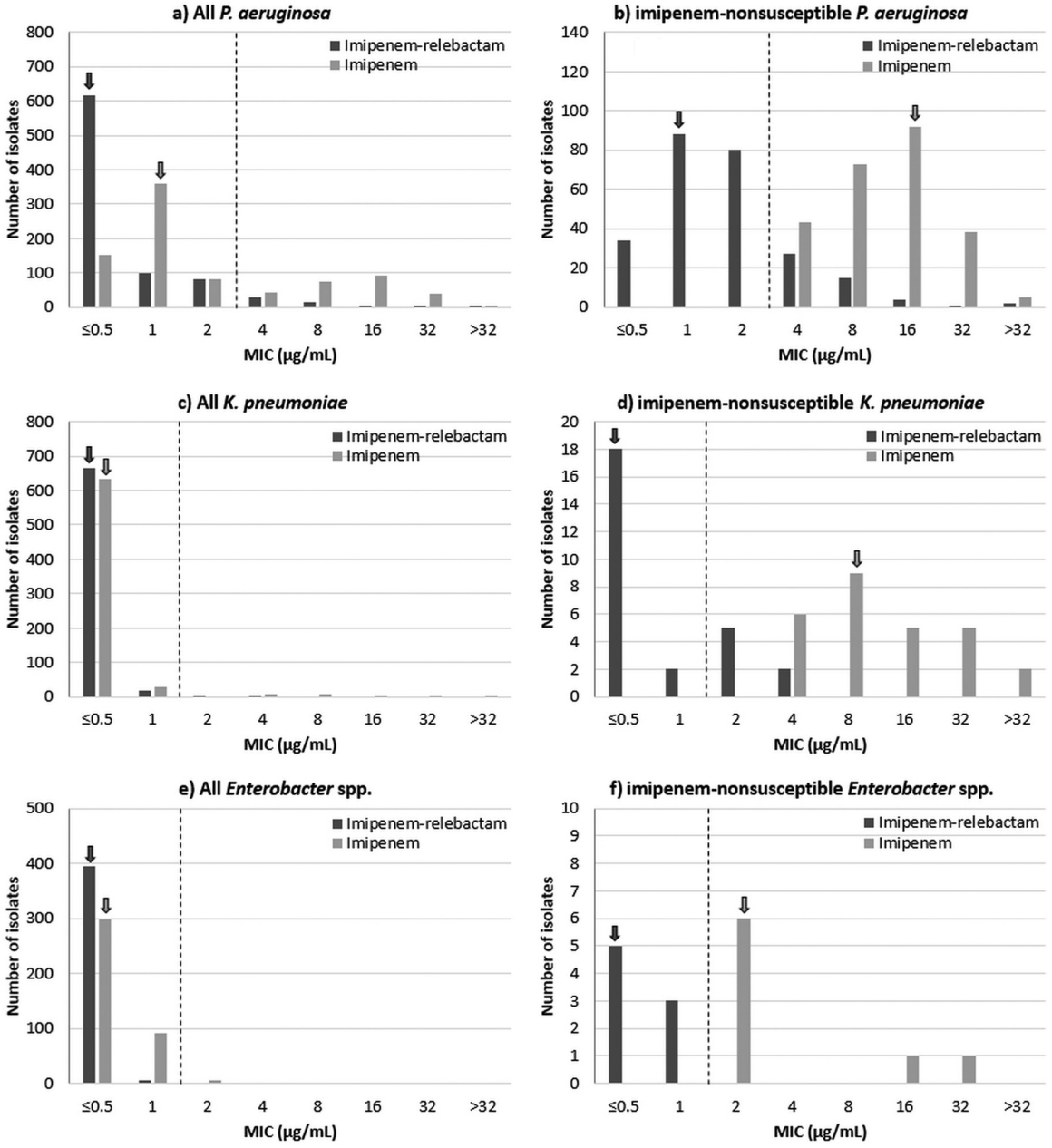

FIG 1 Effects of relebactam on the distributions of MICs for imipenem against 845 *P. aeruginosa* isolates (a), 251 imipenem-nonsusceptible *P. aeruginosa* isolates (b), 689 *K. pneumoniae* isolates (c), 27 imipenem-nonsusceptible *K. pneumoniae* isolates (d), 399 *Enterobacter* species isolates (e), and 8 imipenem-nonsusceptible *Enterobacter* species isolates (f). Arrows indicate the modes of the MIC distributions. Dashed lines represent the imipenem CLSI susceptibility breakpoints of ≤2 μg/ml for *P. aeruginosa* and ≤1 μg/ml for *Enterobacteriaceae*.

imipenem-hydrolyzing AmpC that is ubiquitous in that species (9). The propensity for relebactam to inhibit AmpC in *P. aeruginosa* is an important property because only about 10% of isolates produce detectable levels of OprD (9, 20). Geographic differences in the activity of imipenem-relebactam against *P. aeruginosa* may be observed and will be dependent upon geographic differences in the prevalence of metallo-β-lactamases (9).

Livermore et al. reported that at a concentration of 4 μg/ml relebactam reduced imipenem MICs for *Enterobacteriaceae* with KPC-type carbapenemases from 16 to 64 μg/ml to 0.12 to 1 μg/ml and that relebactam restored susceptibility to imipenem for 100% (10/10 isolates) of KPC-producing *Enterobacteriaceae* (9). Lapuebla et al. also reported that relebactam, at a concentration of 4 μg/ml, restored imipenem susceptibility to 97% of *K. pneumoniae* isolates with KPC-type β-lactamases ($n = 111$) and

TABLE 2 Acquired β-lactamases detected in imipenem-nonsusceptible isolates ($n = 286$)[a]

Phenotype/β-lactamase content	No. of isolates (% of phenotype)		
	P. aeruginosa[b] ($n = 251$)	*K. pneumoniae* ($n = 27$)	*Enterobacter* spp.[b] ($n = 8$)
Imipenem-relebactam-susceptible isolates (*n*)	202	20	8
KPC		12 (60.0)	1 (12.5)
KPC + ESBL		6 (30.0)	
KPC + AmpC		2 (10.0)	
ESBL	1 (0.5)		
No acquired β-lactamase detected	201 (99.5)		7 (87.5)
Imipenem-relebactam-nonsusceptible isolates (*n*)	49	7	0
OXA-48-like + ESBL		5 (71.4)	
VIM	2 (4.1)		
VIM + ESBL		1 (14.3)	
GES carbapenemase		1 (14.3)	
No acquired β-lactamase detected	47 (95.9)		

[a]Original spectrum β-lactamases (e.g., TEM-1 and SHV-1) are not included in this analysis.
[b]Intrinsic AmpC β-lactamases common to *P. aeruginosa* and *Enterobacter* spp. are not shown.

restored imipenem susceptibility in a small collection ($n = 7$) of KPC-containing isolates of *Escherichia coli* and *Enterobacter* spp. in which the original imipenem MICs ranging from 0.5 to >16 μg/ml (6/7 isolates were nonsusceptible to imipenem) were lowered to 0.12 to 2 μg/ml (19). Relebactam has also been reported to provide weak potentiation of imipenem activity against some isolates of *K. pneumoniae* with class D OXA-48 enzymes (9). The addition of relebactam at a fixed concentration of 4 μg/ml to imipenem was shown to lower imipenem MICs for 14 isolates of KPC-producing *K. pneumoniae* that also expressed *ramA* or *acrB*, were without frameshift mutations in *ompK35*, or demonstrated altered expression of *ompK36* (19). Among these isolates, the addition of relebactam reduced imipenem MICs at least 8-fold, from 2 to >16 μg/ml to 0.25 to 0.5 μg/ml, against 10 KPC-producing *K. pneumoniae* isolates with elevated expression of *ompK36*; similarly, imipenem MICs were reduced from 4, >16, >16, and >16 μg/ml to 0.5, 2, 2, and 8 μg/ml, respectively, against four KPC-producing isolates with reduced expression of *ompK36* (19). Relebactam also restored the activity of imipenem against a set of imipenem-nonsusceptible (MICs of 2 to 16 μg/ml) isolates of *K. pneumoniae* and *Enterobacter cloacae* that carried ESBLs or AmpC and showed impermeability (9).

A. baumannii is intrinsically resistant to several classes of antimicrobial agents, is frequently multidrug resistant, and is associated with hospitalized patients (21). In this study, relebactam did not increase the percent susceptibility of imipenem against clinical isolates of *A. baumannii* (Table 1). This phenotypic observation was previously made by Lapuebla et al., who also reported that relebactam did not improve the activity of imipenem against isolates of *A. baumannii* that overexpressed AmpC and/or OXA-51 β-lactamase, suggesting that relebactam lacks activity against these enzymes in *A. baumannii* (19). In this study, the rates of resistance to all of the agents tested against *A. baumannii*, including amikacin, were >30%. *A. baumannii* currently remains an infrequent pathogen relative to the prevalence of other ESKAPE pathogens and demonstrates variability in geographic prevalence (21).

Imipenem-relebactam has successfully completed two phase 2 clinical trials for treating complicated intra-abdominal infections and complicated urinary tract infections (ClinicalTrials registration no. NCT01506271 and NCT01505634, respectively), is currently in phase 3 development for the treatment of imipenem-resistant Gram-negative infections, including hospital-acquired bacterial pneumonia, ventilator-associated bacterial pneumonia, complicated intra-abdominal infections, and complicated urinary tract infections (ClinicalTrials registration no. NCT02452047), and is in a second trial for patients with hospital-acquired or ventilator-associated bacterial pneumonia (ClinicalTrials registration no. NCT02493764).

We conclude that Gram-negative ESKAPE pathogens isolated from patients in the United States in 2015 demonstrated reduced *in vitro* susceptibility to advanced-generation

cephalosporins (cefepime, ceftazidime, and ceftriaxone), piperacillin-tazobactam, and fluoroquinolones (levofloxacin), and that relebactam, the non-β-lactam bicyclic diazabicyclooctane β-lactamase inhibitor, demonstrated a strong propensity to restore the *in vitro* activity of imipenem against carbapenem-nonsusceptible isolates of the ESKAPE pathogens, *P. aeruginosa*, *K. pneumoniae*, and *Enterobacter* spp. Further development of imipenem-relebactam is warranted as it would provide clinicians with a much needed option for treating infections caused by carbapenem-resistant *Enterobacteriaceae* and *P. aeruginosa* beyond therapy with polymyxins, tigecycline, or an aminoglycoside, all of which have been associated with significant morbidities, including nephrotoxicity, vestibular ototoxicity, and cholestatic jaundice.

MATERIALS AND METHODS

Bacterial isolates. In 2015, 21 hospital laboratories across 15 states in the United States (California, Colorado, Florida, Georgia, Illinois, Indiana, Iowa, Kentucky, Michigan, New York, North Carolina, Ohio, Pennsylvania, Washington, and Wisconsin) participated in the SMART global surveillance program. Each participating laboratory was requested to submit consecutive Gram-negative aerobic or facultative pathogens cultured from the clinical specimens of patients with intra-abdominal ($n = 100$), urinary tract ($n = 50$), and lower respiratory ($n = 100$) infections to International Health Management Associates, Inc. ([IHMA] Schaumburg, IL, USA), which acted as the central testing laboratory for this study. The 21 participating laboratories collected 4,367 isolates of Gram-negative bacilli from intra-abdominal infections ($n = 1,545$), urinary tract infections ($n = 1,033$), lower respiratory tract infections ($n = 1,764$), and unspecified infection sites ($n = 25$). Gram-negative ESKAPE pathogens (*K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp.) accounted for 38.1% of the intra-abdominal infection isolates, 34.4% of the urinary tract infection isolates, and 59.7% of the lower respiratory infection isolates collected. All of the isolates received by IHMA were reidentified using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) spectrometry (Bruker Daltonics, Billerica, MA, USA).

Antimicrobial susceptibility testing. All antimicrobial susceptibility testing was performed at IHMA using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (22, 23) with custom-made dehydrated Trek Diagnostic Systems panels (Thermo Scientific, Independence, OH). All isolates were tested against imipenem-relebactam, imipenem, amikacin, aztreonam, cefepime, ceftazidime, ceftriaxone, levofloxacin, and piperacillin-tazobactam. Relebactam was tested at a fixed concentration of 4 μg/ml in combination with 2-fold dilutions of imipenem. MICs were interpreted as susceptible, intermediate, or resistant using CLSI breakpoints (22). MICs for imipenem-relebactam were interpreted using imipenem MIC breakpoints for *Enterobacteriaceae* (susceptible, 1 μg/ml; intermediate, 2 μg/ml; resistant, 4 μg/ml) and for *P. aeruginosa* and *Acinetobacter* spp. (susceptible, 2 μg/ml; intermediate, 4 μg/ml; resistant, 8 μg/ml).

Screening for β-lactamase genes. All of the imipenem-nonsusceptible isolates of *P. aeruginosa* ($n = 251$), *K. pneumoniae* ($n = 27$), and *Enterobacter* spp. ($n = 8$) were tested for the presence of genes encoding β-lactamases using published multiplex PCR assays, followed by full-gene DNA sequencing as described previously (24, 25). Specifically, we screened all isolates for genes encoding the metallo-β-lactamases (IMP, VIM, NDM, GIM, and SPM), serine β-lactamases (KPC, OXA-48-like, and GES), ESBLs (SHV, TEM, CTX-M, VEB, PER, and GES), acquired AmpC β-lactamases (ACC, ACT, CMY, DHA, FOX, MIR, MOX), and PDC (*P. aeruginosa* only). Imipenem-nonsusceptible isolates of *A. baumannii* were not molecularly characterized because the addition of relebactam to imipenem did not meaningfully improve its *in vitro* activity against *A. baumannii* and isolates were frequently nonsusceptible to imipenem-relebactam.

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